

exhaustion. Several compounds, including chlordecone (CD) and the s-chlorothiazine herbicide atrazine (ATRA), first induce lengthened cycles and then PE. We developed a pharmacodynamic estrous cycle (PD-EC) model for the S-D rat that focuses primarily on interactions between LH and E2. E2 has positive and negative feedback effects on LH release from the pituitary but produces hypothalamic toxicity. E2 also mediates transcription in the HYPO leading to synaptic remodeling. Our model assumes that failure of the LH surge and ovulation ensue when cumulative-E2 toxicity leads to insufficient HYPO-E2 receptor reserve to accomplish remodeling in the intercycle period. The model was calibrated by examining data on altered cycle characteristics and PE induced by ATRA. The most intriguing model-derived insight was the prediction that both weak functional agonists (i.e., CD) and weak functional antagonists (i.e., the triazine herbicides) could lead to early onset PE. The behavior occurs because of interactions between organs with little receptor reserve, e.g., HYPO, and organs with spare receptors, the ovaries, uterus, breasts, etc. Weak functional agonists and antagonists preferentially affect E2-responses in receptor poor tissues (the LH surge mechanism in the HYPO) without overt effects on receptor-rich organs. This PD-EC model may be useful in evaluating toxic endpoints caused by various endocrine modulators, such as xenobiotic-induced mammary tumors in S-D rats, and for determining whether threshold doses are likely to be associated with those responses. (This work was supported by CIBA-Crop Protection).

597 DEVELOPING PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PB-PK) MODELS FOR MANGANESE AND IRON

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Iron (Fe) and manganese (Mn) are essential transition series elements which exist in the body in the +2 and +3 valences states. Fe transport throughout the body is regulated by a carrier protein, transferrin (Tr), and tissue storage of that portion of Fe not involved in protein function or O₂ storage occurs in a ferritin (Ferr), a specific Fe-binding protein, and hemosiderin. To a large extent, Mn disposition follows the Fe pathways. Despite tight control, both metals are toxic in overload conditions. We have developed PB-PK models of Fe and Mn transport to aid in interpreting nutritional and toxicological studies with these elements. The model includes Mn kinetics for different valence states and for inhalation, ingestion, and intraperitoneal and intravenous dosing routes. An Fe model was also developed to form the basis of considering interactions between Fe status and Mn disposition. In the model, Mn⁺² is oxidized in the blood and tissues to Mn⁺³ and is also eliminated in the bile. This fecal elimination of Mn⁺² is the only loss route. The model was used to successfully describe time-course data in the literature from rats, monkeys, and humans exposed to Mn⁺², Mn⁺³, and mixed valence compounds. Deep tissue stores of Mn, presumably essential Mn in enzymes, do not equilibrate rapidly with newly absorbed Mn leading to biphasic disposition kinetics. In the homeostatic region, intestinal uptake, and blood uptake from intestinal cells are closely adjusted to elimination. In overload, the excess intake intake associated with both saturable and first-order uptake processes is not eliminated sufficiently rapidly, resulting in accumulation of free or loosely bound Mn in target tissues and in toxicity. One suggestion from this preliminary model is that Mn overload may result from less avid binding of excess Mn to Ferr compared to Ferr binding of Fe. With refinement, these models should be useful in developing non-cancer exposure guidelines for Mn which acknowledges its essentiality.

598 BIOLOGICALLY-BASED RISK ASSESSMENT MODELS FOR DIOXIN HEPATOCARCINOGENESIS SUPPORT NON-LINEAR LOW DOSE EXTRAPOLATIONS

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Studies with dioxin and other liver tumor promoters support a negative selection mechanism of tumor promotion. First, dioxin acts as a weak mitogen; the body then adapts by elaborating mito-inhibitory growth factors; finally, specific liver cells, unresponsive to mito-inhibition, grow out to clones of altered cells. We linked physiologically-based pharmacokinetic (PB-PK), gene induction, and clonal growth models to describe the dioxin tumor promotion dose-response curve. The PB-PK/gene induction portion accounts for (1) dioxin binding to the Ah receptor and to cytochrome P4501A2, both of which are induced by dioxin, and (2) induction of cytochromes and alterations of cell growth parameters in the liver by Ah receptor-dioxin-DNA

complexes in a regional and dose-dependent manner. The gene induction model provides input to the clone growth model where 2 populations of initiated cells respond differentially. Growth of one population is suppressed by the mito-inhibitory environment; growth of the second, unaffected by the anti-proliferative milieu, is enhanced at higher, hepatotoxic doses of dioxin. In our model, mito-suppression is correlated with centrilobular induction of proteins; clonal expansion of resistant cells is correlated with the panlobular induction at higher doses. We used the model to analyze liver tumor promotion/protein induction data from sub-chronic studies from 1 to 125 ng dioxin/kg/day. The simulated dose-response curves are U-shaped with a region where dioxin actually reduces the growth of altered cells compared to controls. Based on this highly-nonlinear behavior, the risk assessment for dioxin hepatocarcinogenesis should be based on a margin-of-exposure factor (100 to 1000) and corrections for differences in kinetics between animals and humans. Linear extrapolations do not appear warranted for this endpoint. (Supported in part by the American Forest and Paper Association).

599 ANGLER-CAUGHT FISH CONSUMPTION DISTRIBUTIONS FOR ANGLERS AND THE GENERAL PUBLIC FOR IMPROVED PROBABILISTIC RISK ASSESSMENT

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Probabilistic risk assessment requires distributions of various inputs to characterize their inherent variability. Consumption of angler caught fish can be a significant exposure pathway particularly for lipophilic toxicants. The Santa Monica Bay Fish Consumption Study (1994) is a well conducted survey addressing a diverse California angler population. SAS® was used to derive distributions from the raw data from this study. Systat® was used to develop the associated probability plots. The fish consumption distribution derived from this study reveals fish consumption is highly skewed indicating a lognormal distribution. The mean, standard deviation, skewness, and kurtosis are 49.6 g/day, 81.7 g/day, 5.83, and 50.0, respectively. This distribution indicates that anglers can be sensitive subpopulation because of their high consumption rates relative to the general population. In order to determine risk to the general population from angler-caught fish consumption a second distribution was derived from the angler fish consumption. The number of combined fresh and salt water fishing licenses was determined and divided by the population over 16 (licensed anglers/population). The fraction of anglers (0.06) was multiplied times the average California household of 2.7 to yield the fraction (0.16) of the California population who consumed angler-caught fish. This fraction was multiplied times the angler fish consumption distribution to yield an angler-caught fish consumption distribution for the general public. The mean, standard deviation, skewness, and kurtosis are 8.73 g/day, 19.6 g/day, 9.59, and 129.3, respectively. These distributions allow a more accurate determination of the risk to anglers and the general population from consumption of angler-caught fish. These risks have been underestimated using standard point estimates of fish consumption.

600 ASSESSMENT OF AIRBORNE HEXAVALENT CHROMIUM IN THE HOME FOLLOWING USE OF CONTAMINATED TAPWATER

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Field studies were conducted to estimate the plausible uptake of hexavalent chromium [Cr(VI)] aerosols inhaled during indoor residential use of a shower and an evaporative cooler supplied with water containing Cr(VI). In the evaporative cooler study, water concentrations of 20 mg Cr(VI)/L did not produce an increased concentration of airborne Cr(VI) inside the home. In the shower study, the average airborne concentrations of Cr(VI) aerosols at breathing zone height ranged from 87 to 324 ng Cr(VI)/m³ when the water concentration of Cr(VI) was 0.89 to 11.5 mg/L. The Cr(VI) concentration in air was directly correlated to water concentration. The lifetime average daily doses and incremental cancer risk estimates corresponding to 30-year residential exposures were calculated using the measurements in this study and published exposure guidelines. The plausible upperbound lifetime cancer risk associated with continuous exposure to "background" Cr(VI) in outdoor air was estimated at 6.9 per million for a person exposed from age 0-30, and 4.0 per million for age 30-60. Similarly, estimated upperbound cancer risks due to inhaling shower aerosols (10 minutes per day, every day) from water containing 2-10 mg Cr(VI)/L over the same exposure period ranged

from 0.9 to 5.5 per million. Our calculations demonstrate that shower aerosols do not contribute appreciably to background Cr(VI) exposures and risks at water concentrations up to 10 mg/L.

601 CHRONIC REPRODUCTIVE EFFECTS ARE SEEN IN DAPHNIA MAGNA EXPOSED TO A NON-STEROIDAL AROMATASE INHIBITOR, CGS 16949

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Sponsor: AT Eldefrawi

D. magna exhibits multiple P450 enzyme activities which can hydroxylate exogenous testosterone (Baldwin and LeBlanc, 1994). The capacity to produce estrogens via aromatase, an ancient cytochrome P450 which converts androgens to estrogens, has not been demonstrated. My preliminary results indicated that aromatase activity is present in adult *D. magna*. Subsequently, I performed a 28-day chronic *D. magna* reproduction assay to assess the effects of exposure to CGS 16949, a non-steroidal aromatase inhibitor used in human breast cancer treatment. A concentration of 24 μ M CGS (LC₅₀ = 250 μ M) delayed the onset of reproduction by 1–2 days in the first generation and caused a significant decrease (one-way ANOVA, $p=0.05$) in the number of neonates produced. A further 28-day chronic assay using neonates from the second brood with continued exposure to the same concentrations of CGS 16949 indicated that, while total numbers of neonates produced by treated animals was not different from controls, exposure to 24 μ M CGS caused a marked phase change in the reproductive cycle. No significant difference in total protein measured per milligram dry weight was seen in either the first or second generation. These results suggest a role for estrogens in the reproductive cycle, but the mechanisms of action and consequences are unknown.

602 EVIDENCE OF GLUTATHIONE S-TRANSFERASE π IN HUMAN UTERINE TISSUE

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The glutathione S-transferase (GST) π -isoform is a cytosolic enzyme, principally involved in the detoxification of reactive metabolites. It is well established that the risks of either cervical or endometrial cancers are linked to cigarette smoking. No reports exist on the localization of GST isoforms in human cervical or endometrial tissues. We report here immunohistochemical evidence for the localization of GST π in these regions. We have studied uterine tissue obtained from normal and endometrial adenocarcinoma patients. Sections containing cervix ($n=12$), endometrium ($n=11$) and adjacent tumor regions ($n=5$) were prepared from paraffin embedded, archival tissue blocks fixed in formalin. Tissues were stained by the avidin-biotin complex method with monoclonal antibodies against placental GST π . Semi-quantitative staining intensity was determined by microscopic examination of the tissues. Significant staining intensity was observed in the superficial squamous epithelial cells in 100% of the normal cervix which decreased in magnitude towards minimal staining in the basal cells. This pattern was not different in the cervix from the endometrial cancer patients, however predominantly nuclear staining was observed in the cervix from the cancer patients. This is the first report of immunohistochemical evidence of GST π in the squamous region of human cervix. Furthermore there seems to be a transition from the cytosolic to nuclear regions in this cell type for cancer patients. The staining intensity in the endometrial glands was minimal in both the normal and cancer patients. However significantly increased staining was observed in the tumor regions, indicating increased expression of GST π in tumor tissues.

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603 PREPUBERTAL INSENSITIVITY TO CARBENDAZIM-INDUCED TESTICULAR TOXICITY

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Previously we have shown that the benomyl (BNL) metabolite carbendazim (CBZ) and not BNL itself is the most likely mediator of BNL-induced testicular toxicity. CBZ is also a potent inhibitor of testicular microtubule assembly and this action is thought to underlie testicular toxicity. Literature reports have indicated that prepubertal animals lack sensitivity to testicular damage after BNL. The present study has utilized CBZ to investigate hypotheses which could explain prepubertal insensitivity to BNL/CBZ. In *in vivo* studies, CBZ (100 mg/kg, ip) was administered to postpubertal (90–110 days) and to prepubertal (30–35 days) rats. Twenty-four hours later, testes were

collected and prepared for histopathological evaluations. CBZ caused little testicular damage in prepubertal rats but in adult rats, CBZ treatment resulted in sloughing of the seminiferous epithelium. When the inhibitory effect of CBZ on prepubertal testicular microtubule assembly was compared to postpubertal, the IC₅₀ was actually lower in the prepubertal than postpubertal animals. By isoelectric focusing, no apparent differences in the pattern of β -tubulin isotypes was observed. Pharmacokinetic studies revealed the most likely reason for the differential sensitivity of young vs. adult animals. High levels of CBZ were found in the adult testes yet none were detected in testes from young animals 1 hr and 2 hr after administration of CBZ (164 mg/kg, ip). These data suggest the age-dependent differences in susceptibility to toxicity are due to pharmacokinetic differences and do not reflect a differential responsiveness of testicular cells. Supported by NIH grant #ES05707.

604 THE HOBSON TRACKER AS A SUCCESSFUL TOOL IN REGULATORY TOXICITY STUDIES FOR THE EVALUATION OF RAT FERTILITY

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The Hobson Tracker enables continuous tracking of motile sperm over an unlimited period of time. The system has been validated for use in a GLP environment and has been shown to be useful in the assessment of fertility when a known anti-fertility agent is used (α -chlorohydrin). In particular changes in VSL have appeared to be of interest. Consequently the system has been used as a tool in a number of regulatory reproductive studies where fertility is being assessed in the conventional manner. In 5 of the 6 studies which have been evaluated, the fertility levels as measured by number of pregnant animals, corpora lutea, conception rates and live embryos were normal, as was the Hobson Tracker data. However, in the 6th there was a significant reduction in both VSL and semen concentration in the high dose group. When the fertility data was assessed (independently) there was a noticeable reduction in conception rates and live embryos. These results lend support to the argument that CASA is of value as a tool for predicting reductions in fertility levels. An enlarged data pool may produce sufficient evidence of the alteration in sperm motility parameters for a reduction/cessation of rodent fertility studies and their replacement with CASA.

605 EFFECT OF SUBCHRONIC ORAL MTBE EXPOSURE ON REPRODUCTIVE ENDOCRINE FUNCTION AND METABOLISM IN MALE RATS

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Methyl-t-butyl ether (MTBE), a widely-used motor fuel oxygenate, caused Leydig cell tumors in chronic studies of rats exposed to 8000 ppm by inhalation or 1000 mg/kg by gavage. MTBE also produced renal tumors in male rats and liver tumors in female mice, and induces P-450 in rats and mice. To explore the possibility that high doses of MTBE cause tumors as a result of an imbalance of reproductive hormone levels, male Sprague-Dawley rats were gavaged with MTBE in corn oil for one month. MTBE doses were initially 1500 and 1000 mg/kg, and were lowered to 1000 and 750 mg/kg to maintain body weight. Serum testosterone decreased in the higher MTBE dose group within hours after the first treatment ($p<0.05$), but was no different from controls at the time of sacrifice. Liver total P-450 was slightly elevated in the higher dose group after one month of treatment ($p<0.05$). These effects were not observed at the 750 mg/kg dose. Liver and androgen-dependent organ weights were unchanged. An immediate, transient decrease in serum testosterone is more consistent with a direct effect on the hypothalamic-pituitary-gonadal axis or stress of treatment. Biologically significant long-term changes in steroid metabolism might occur if P-450 induction is sustained.

606 MONO-(2-ETHYLHEXYL) PHTHALATE RAPIDLY ALTERS TESTICULAR GERM CELL APOPTOSIS

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Phthalic acid esters, used as plasticizers in food packaging and medical devices, reduce fertility and induce testicular atrophy in laboratory animals. The Sertoli cell of the seminiferous epithelium is recognized as the primary cellular target for phthalate-induced testicular injury. Phthalate-induced alterations in Sertoli cells lead to a rapid disruption of the Sertoli-germ cell physical